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Increased delay between harvest and processing

- effect on protein paste yield and composition

Production of protein samples

Red clover and perennial ryegrass were harvested and cut. After harvest the cut plants were laid in a pile in a covered barn.

Red clover was harvested in August early morning on a rainy day. Perennial ryegrass was harvested in August early morning on a dry day.

Immediately after harvest and then 3, 6, 8, and 24 hours after harvest, a representative sample was collected from the piles.

The plants were processed in a lab-scale screwpress (Angle 8500s). The resulting juice was acidified with phosphorous acid until pH 4, left over night in the cold room and subsequently centrifuged (2000 g, 10 min, 4 degrees). Samples were freeze-dried and analyzed.

Observations

Storage of harvested cut samples in a pile for prolonged time also increased the pile temperature. After 24 hours, the temperature of the pile was increased substantially and the pile was steaming. Storage of the plant for 24 hours resulted in a plant juice from red clover with a fermented odor equated with a drop in juice pH, whereas the juice from ryegrass smelled decomposed and pH increased.

Protein content and composition in protein paste

The content of dry matter (DM) and nitrogen (N) was measured in all samples. The DM contents was determined in duplicates on freeze-dried material by drying at 103°C for two hours. Nitrogen (N) was analysed by the Dumas procedure using thermal conductivity after complete combustion at 1300°C in pure oxygen. Crude protein (CP) was calculated as N multiplied with 6.25. Amino acids in the samples were determined using the EEC (European Economic Community) methods (98/64/EC and 2000/45/EC).

The DM content of the unprocessed red clover plant is shown in figure 1, whereas that for perennial ryegrass is shown in figure 2.

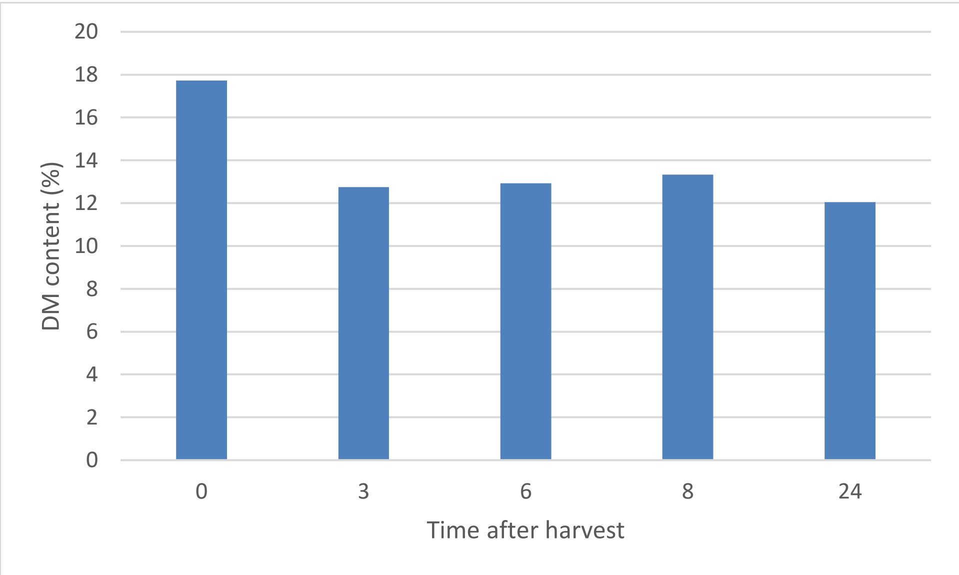

Figure 1 Dry matter content of red clover plant

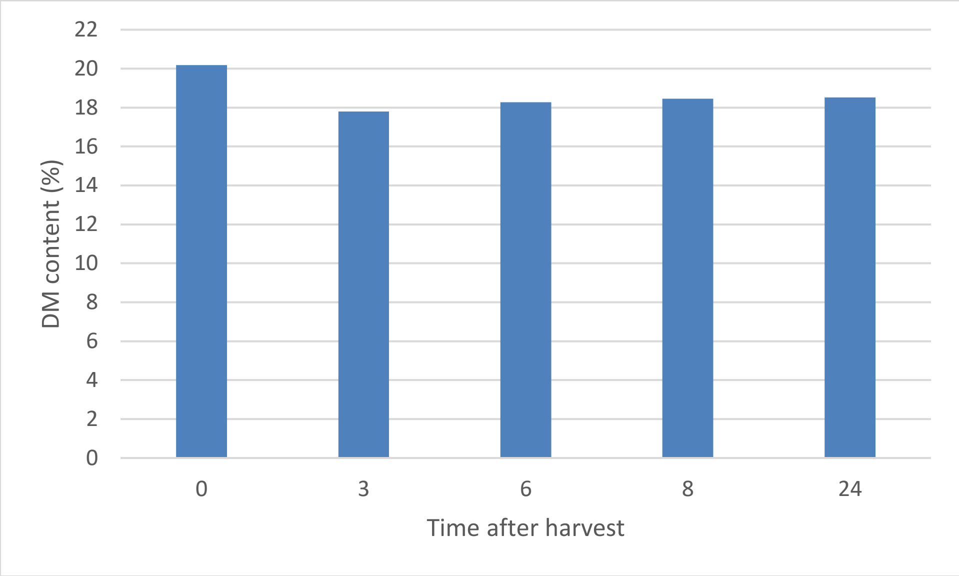

Figure 2 Dry matter content of perennial ryegrass plant (freeze-dried DM content)

The newly harvested red clover plant had a DM content of 17.7 %, which decreased with increasing storage time. After storage for 24 hours, the red clover had a DM content of 12%. Perennial ryegrass had a DM content of 20.2% at time of harvest decreasing slightly to 18.5% after 24 hours storage. Normally, storage of harvested plants (e.g. wilting for silage) will increase DM due to water loss. As the harvested plants is this study were not exposed to water during storage, the decreased DM is expected to be a result of heterogeneous sampling and low sample size.

The DM of the produced protein paste ranged from 17-21% (data not shown) which was lower than previous findings [1, 2]. The CP content (DM-based) of protein paste produced from red clover and perennial ryegrass is shown in Figure 3.

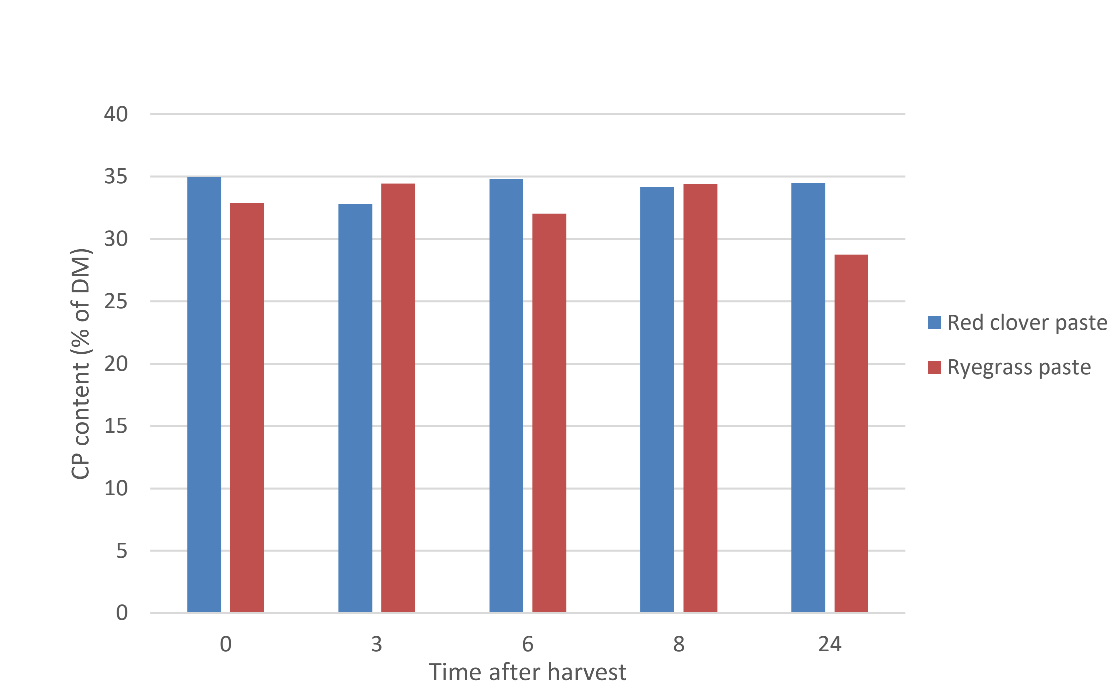

Figure 3 Crude protein (CP) content of protein paste from perennial ryegrass and red clover produced with increased delay from harvest to processing

Immediately after harvest, at time=0, the crude protein content of red clover paste was 35% whereas that of perennial ryegrass was 33%. These results are comparable to earlier findings [1]. Increasing the delay between harvest and processing did not have major effects on CP content of the red clover paste, however after 24 hours storage, the CP content of the ryegrass paste was reduced to 29%.

The CP content is calculated from analysis of nitrogen, and will be constituted of all nitrogen-containing components, including non-protein bound nitrogen. To determine the content of real protein, the amino acid content was determined, as was the composition to determine any storage effects on individual amino acids (figure 4 and 5).

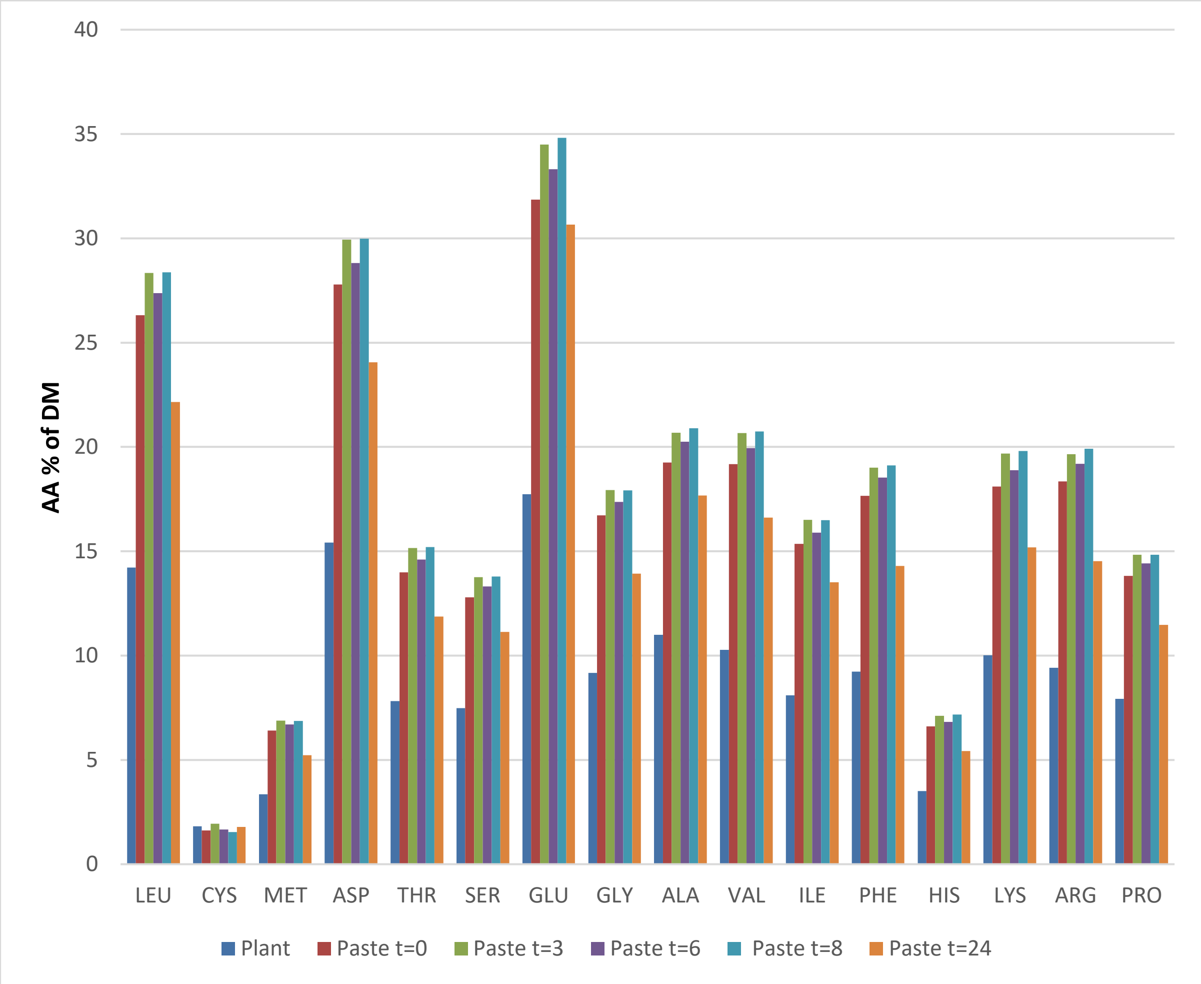

Figure 4 Amino acid composition in perennial ryegrass plant and protein paste produced after storage of plant for 0,3,6,8, and 24 hours. Expressed on dry matter basis

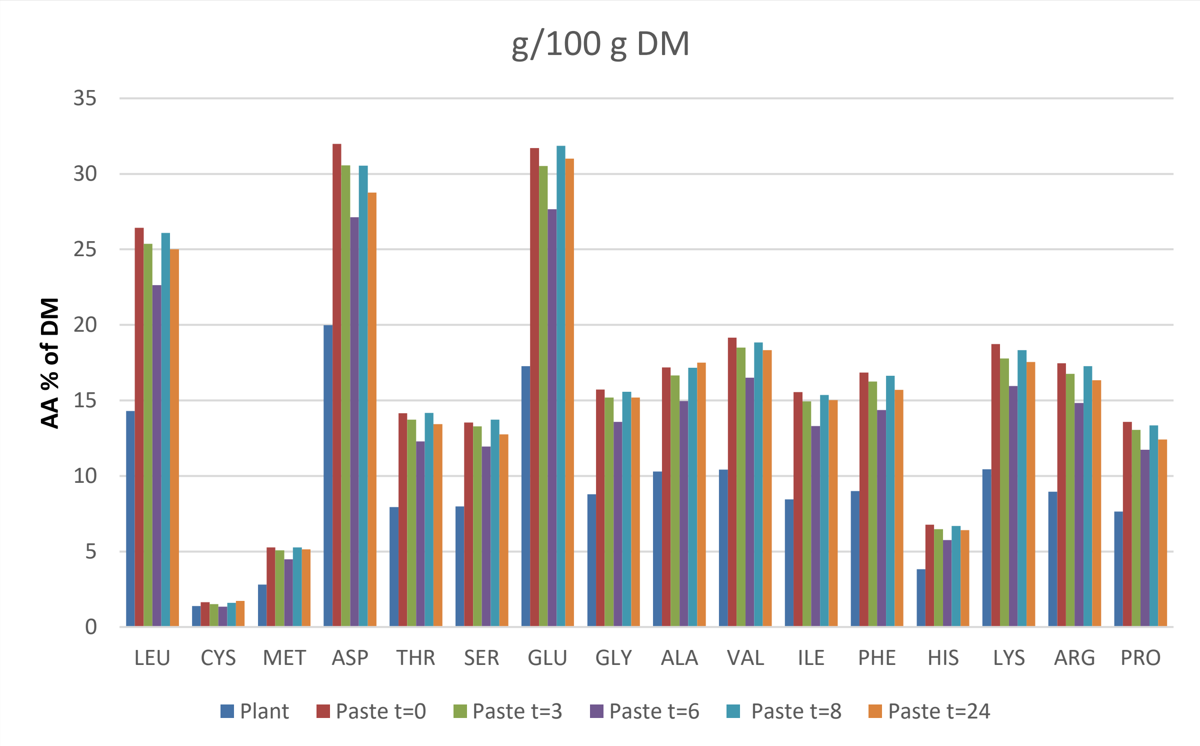

Figure 5 Amino acid composition in red clover plant and protein paste produced after storage of plant for 0,3,6,8, and 24 hours. Expressed on dry matter basis

Compared to the content of amino acids in the unprocessed plant, the content of all amino acids were higher in the produced protein paste when expressed on a DM basis. This is expected, as during acidification, the majority of protein-bound amino acids will be distributed to the protein paste whereas other small components will be removed into the brown juice. The consequence of this is a concentration of amino acids in the paste. For both red clover and perennial ryegrass it can be seen, that the content of the individual amino acids is relatively stable during storage, indicating that the composition of the precipitated protein does not alter due to prolonged delay between harvest and processing. The same can be seen, when expressing the amino acid composition on a CP-basis (figure 6 and 7),

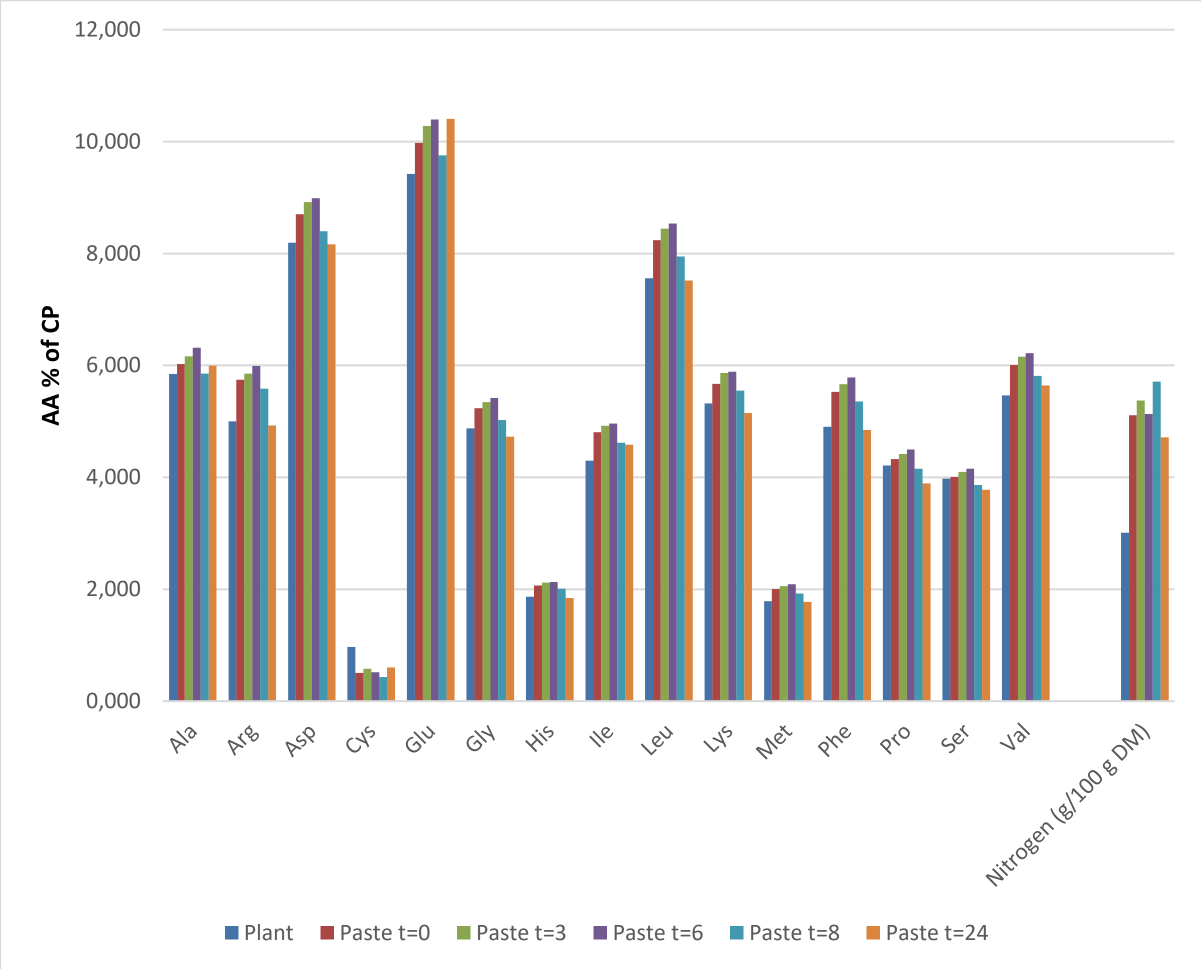

Figure 6 Amino acid composition in perennial ryegrass plant and protein paste produced after storage of plant for 0,3,6,8, and 24 hours. Expressed on crude protein basis.

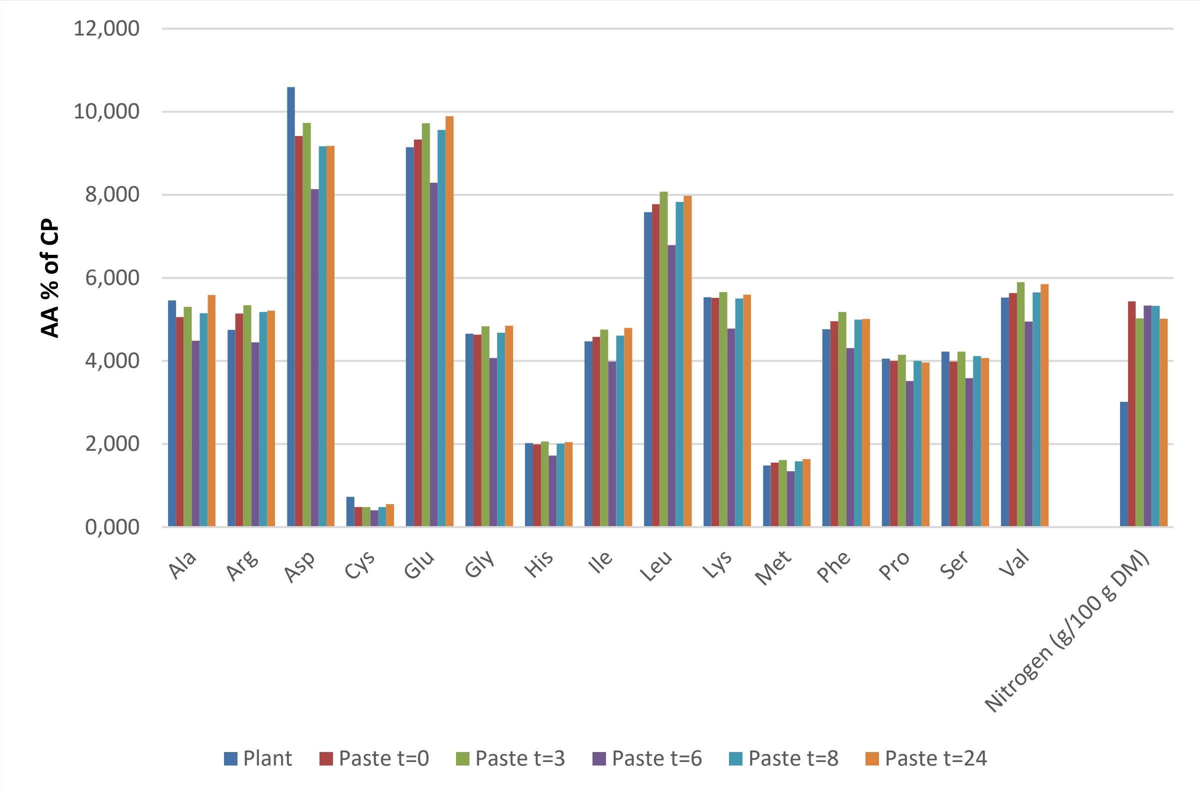

Figure 7 Amino acid composition in red clover plant and protein paste produced after storage of plant for 0,3,6,8, and 24 hours. Expressed on crude protein basis

Protein yield

An important issue when considering using green biomass as source for protein production is the yield, e.g. the proportion of the plant protein ending up in the protein paste. This was determined in the current study (figure 8).

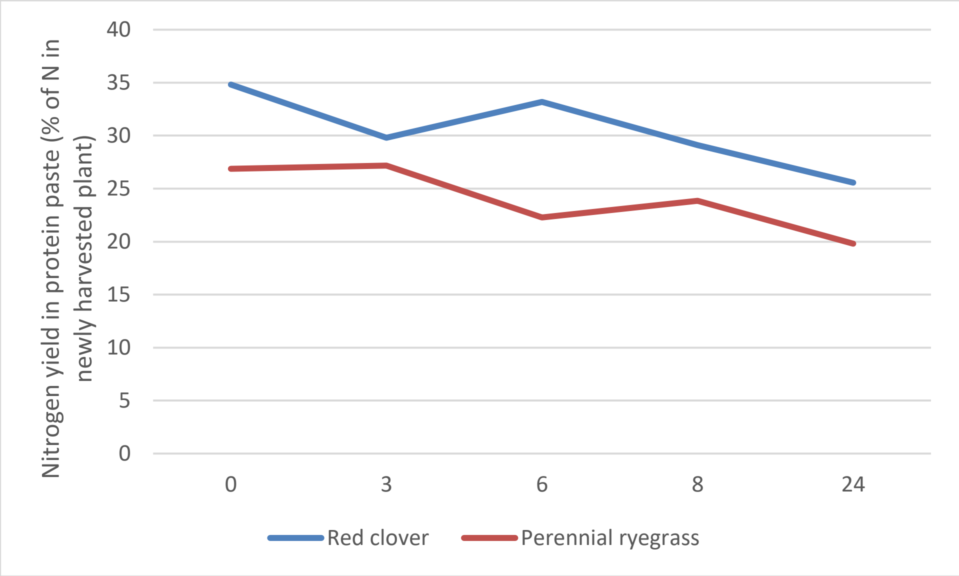

Figure 8 Nitrogen yield in protein paste from red clover and perennial ryegrass expressed as percentage of the nitrogen in the newly harvested plant.

Processing red clover immediately after harvest resulted in precipitation of 35% of the plant nitrogen into the protein paste. For perennial ryegrass, the yield was 27%. Yield is affected by many factors, among these the DM content of the plant. It is know that protein follows the extracted water, and hence and increased plant DM content would subsequently lead to lower yield. Storage of both red clover and perennial ryegrass decreased the nitrogen yield. For red clover, the yield after 24 hours was 25% whereas for perennial ryegrass it was 20%. These results confirm that the plant DM results in figure 1 is likely to be caused by low sample size and not from an actual decreased plant DM upon storage. Another factor, which influenced the extractability is the fiber content of the plants. The difference between yield in red clover and perennial ryegrass may therefore be a question of a higher fiber content in the perennial ryegrass. Proteins will be bound directly to or retained by the fiber network. As the fiber content is expected to be higher in the perennial ryegrass, a higher proportion of the plant protein will be associated with the fibers and will upon processing be distributed to the fibrous pulp leading to a lower yield. The decrease in yield was most pronounced in red clover, which may be caused by the high proportion of oxidative enzymes present in red clover cross-binding protein to fibers and hence increasing the proportion of protein distributed to the pulp.

Volatile fatty acids

The contents of selected volatile fatty acids (VFA), free sugars, lactic acids and other organic acids were analyzed by HPLC and gas chromatography (GC). Measurements were done on the plant juice, the protein paste and the residual brown juice. For perennial ryegrass, the results can be seen in Table 1, and for red clover in Table 2.

Table 1. Volatile fatty acid and free sugars in perennial ryegrass samples

			Component							
Fraction	**Storage time**	**Cellobiose**	**Glucose**	**Fructose/Xylose**	**Lactic acid**	**Citric acid**	**Succinic acid**	**Acetic acid**	**Butytic acid**	**Valeric acid**
Juice	0	0.96	7.25	18.16		1.94	0.88	1.10	0.19	0.05
	3	0.77	6.00	15.55		2.09	0.75	0.67	0.17	0.10
	6	0.70	6.19	17.44		1.86	1.50	0.70	0.18	0.10
	8	0.71	6.06	16.26		1.93	1.50	0.48	0.15	0.05
	24	0.60	3.14	11.94	0.28	1.81	1.31	0.75	0.15	0.11
Protein paste	0		5.09	9.81			0.61	2.85		
	3		4.44	8.31		1.38	0.66	2.89		
	6		4.60	9.90		1.14	1.12	2.93		
	8		4.38	8.67		1.23	1.10	2.96		
	24		2.05	6.37		1.08	0.81	2.96		
Brown juice	0	0.64	7.12	17.49		2.01	1.06	0.47	0.16	0.11
	3	0.80	8.83	14.66		1.67	0.92	0.43	0.14	0.12
	6	0.41	6.04	16.59		1.71	1.39	0.45	0.14	
	8	0.49	5.83	15.80		1.88	1.39	0.48	0.14	0.09
	24	0.13	8.75	12.03		1.51	1.17	0.62		0.09

For juice and supernatant samples, the results are expressed as g/l. For protein paste, the results are expressed as g/kg. Empty cells indicate a measurement of 0 g/l /g/kg

Table 2. Volatile fatty acid and free sugars in red clover samples

			Component							
Fraction	**Storage time**	**Cellobiose**	**Glucose**	**Fructose/Xylose**	**Lactic acid**	**Citric acid**	**Succinic acid**	**Acetic acid**	**Butytic acid**	**Valeric acid**
Juice	0	0.61	8.48	4.04		1.55		0.84		
	3	0.60	7.45	3.52		1.37		0.78		
	6	0.63	7.31	3.71		1.53		0.82		
	8	0.61	6.80	3.50		1.59		0.89		
	24		2.09	0.81	2.17	0.60		2.47		
Protein paste	0		6.97	3.09				3.56		
	3		6.06	2.99				2.99		
	6		5.20	2.79				2.94		
	8									
	24		1.32	0.71				3.64		
Brown juice	0		7.18	3.34			1.30	0.76		
	3		6.82	3.16			1.20	0.92		
	6		5.28	2.55			1.05	0.84		
	8		5.06	2.63			1.14	0.89		
	24		1.87	0.79	2.01		0.74	2.39		

For juice and supernatant samples, the results are expressed as g/l. For protein paste, the results are expressed as g/kg. Empty cells indicate a measurement of 0 g/l /g/kg

Storage of both red clover and perennial ryegrass lead to a decreased content of glucose in the juice samples. Moreover, the content of fructose/xylose also decreased. This suggests that the free sugars are consumed by bacteria. In juice from red clover, there was a measurable amount of lactic acid after 24 hours of storage. This was also seen with juice from perennial ryegrass, which also contained small amounts of buturic acid and valeric acid. The latter two did have change with increased delay between harvest and processing. For red clover, the content of acetic acid was stable until 8 hours of storage. At 24 hours of storage, the content increased. The results are consistent with the observations on a primarily fermented odor in red clover and a more decomposed odor in perennial ryegrass (buturic acid). For the protein pastes, a decrease on the free sugars glucose and fructose was also observed for both plant species. Interestingly, no lactic acid was measured. The content of acetic acid removed stable.

Conclusion

Under the circumstances by which the experiment was conducted, it can be concluded that storage of the plants before processing does not alter the crude protein content of the produced paste, nor does the amino acid composition change. We see minor changes in the VFA and free sugar content, the most notable is the decrease in glucose and fructose.

The most significant effect of increased delay between harvest and processing is the yield, which decreases with as much as 10 percentage point. Even though the composition of the precipitated protein is maintained, the decreased yield is a major challenge in a sustainable and economically feasible large-scale production. From these results, it is clear that plants should be processed as soon as possible, or as minimum within eight hours of harvest.

1. Stødkilde L, Damborg VK, Jørgensen H, Lærke HN, Jensen SK: **Digestibility of fractionated green biomass as protein source for monogastric animals.** *animal* 2019**:**1-9.
2. Stødkilde L, Damborg VK, Jørgensen H, Lærke HN, Jensen SK: **White clover fractions as protein source for monogastrics: dry matter digestibility and protein digestibility-corrected amino acid scores.** *Journal of the Science of Food And Agriculture* 2018, **98:**2557-2563.